Tailor-made transcriptional biosensors for optimizing microbial cell factories

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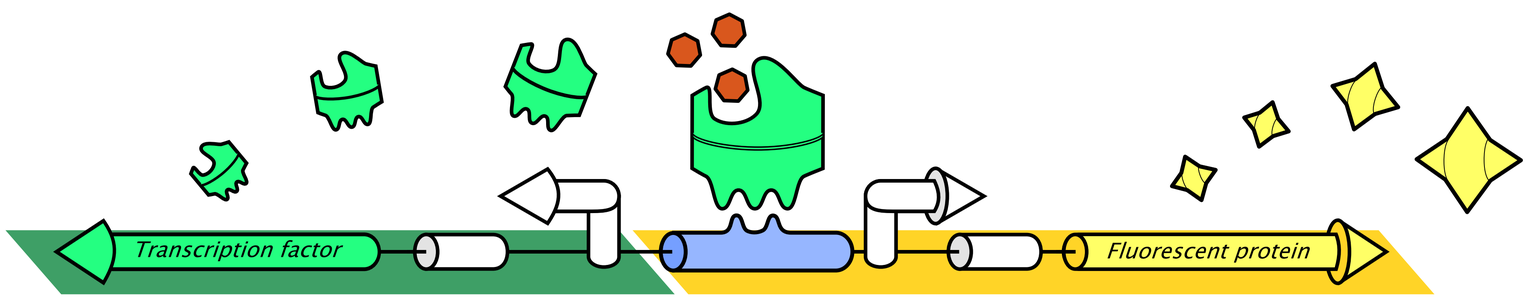
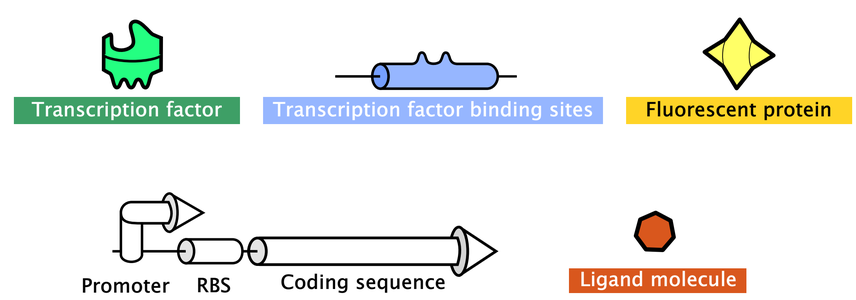
Introduction

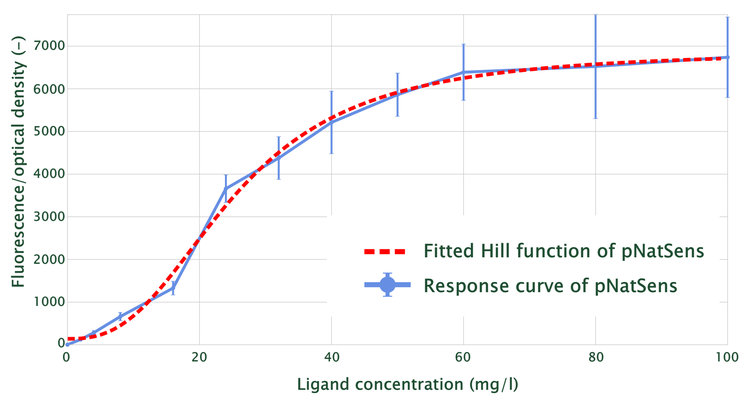
Monitoring the intra- and extracellular environment and adapting metabolic processes accordingly is key to properly address the enormous complexity of today's metabolic engineering questions. To this end, scientists are reprogramming nature's myriad of transcriptional regulatory systems into transcriptional biosensors which are able to detect small molecules and, in response, express output signals. In this manner, biosensors propel key applications, such as high-throughput screening, adaptive laboratory evolution and dynamic pathway control. Hence, transcriptional biosensors bear the potential to augment and accelerate current metabolic engineering strategies, catalyzing vital advances in industrial biotechnology. However, the two key characteristics of these natural biosensor circuits, the response curve and ligand specificity, are typically not in line with the requirements for a real-life biosensor application. Instead, they are evolutionary optimized for specific in vivo conditions. Consequently, engineering efforts are imperative to generate tailor-made biosensors witch custom response curves and ligand specificities. Hence, in this contribution to address these limitations of natural biosensors, several engineering principles were established to create biosensors with custom response curves.

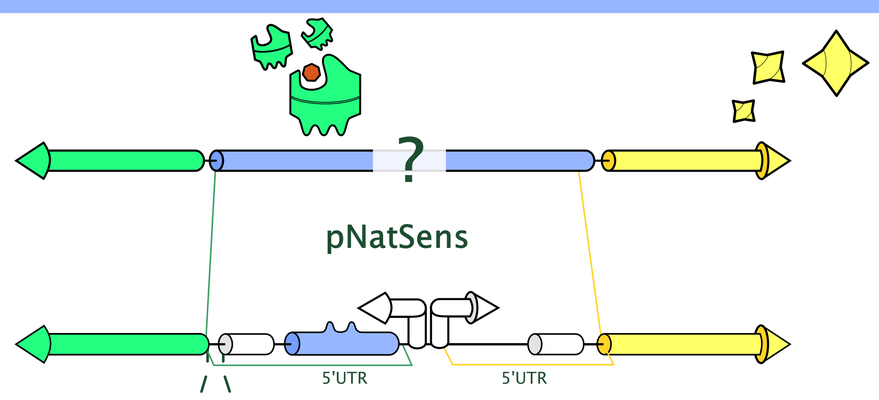




pNatSens – Characterization of native biosensor circuit

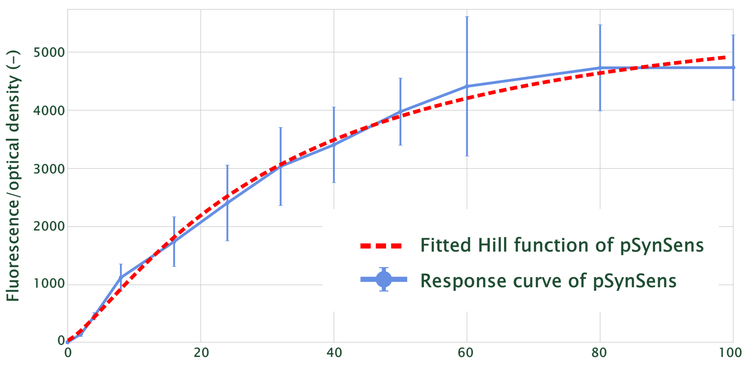
The native biosensor circuit, i.e. pNatSens, was constructed and its response curve characterized. Despite the interesting and useful characteristics of this pNatSens biosensor, such as sensitivity, negligible leaky expression and relevant operational and dynamic range, the lack of any annotation of the intergenic promoter region prevents tailoring this biosensor circuit for custom applications in metabolic engineering strategies. Therefore, comparative sequence analysis was performed to identify engineering target such as, transcription factor binding sites, transcription start sites and core promoter sequences.

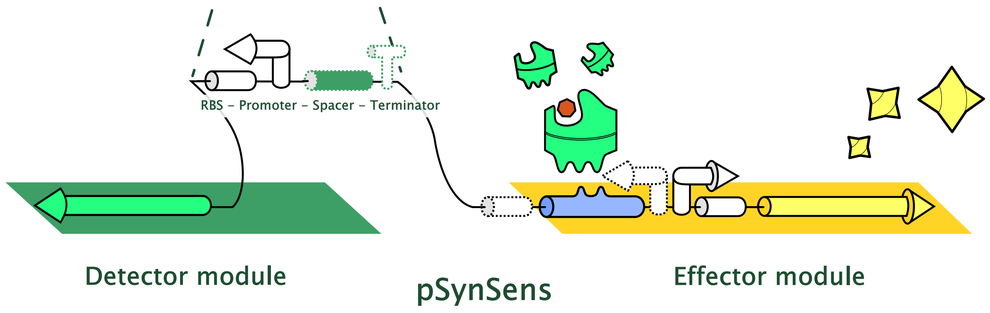




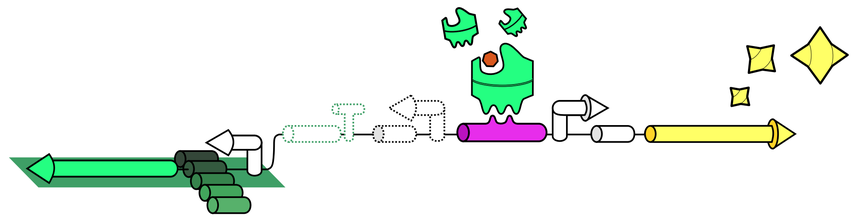
pSynSens – Decoupling of the detector and effector module

With the different genetic parts identified, the path is paved to engineer this biosensor circuit by altering the expression of the detector and effector module, vis-à-vis each other. However, both modules are entwined in this bidirectional intergenic architecture and can not be altered without affecting the opposite module. Therefore, the two modules are decoupled by inserting a terminator and spacer sequence together with a defined promoter and RBS sequence to constitutively express the transcription factor. This synthetic biosensor circuit, i.e. pSynSens, was constructed without loss of functionality in comparison to pNatSens.

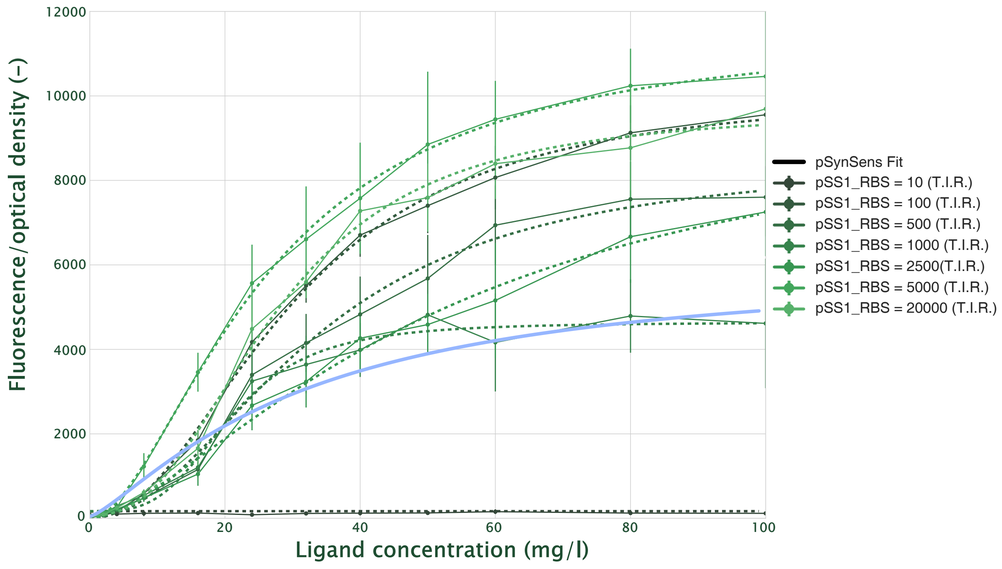




pSynSens1.X – Engineering the detector module



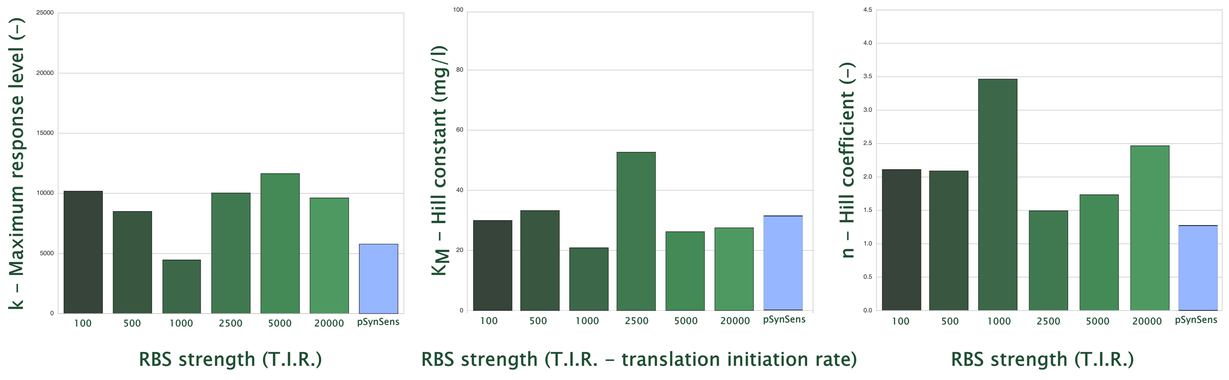
By varying the RBS strength of the detector module, a set of custom biosensors was created with differing characteristics such as, increased operational and dynamic range, varying sensitivity and cooperativity, resulting in distinct Hill parameters. Noteworthy, when targeting the detector module, specific RBS strengths resulted in increased output variability which is atributed to unbalanced levels of transcription factor in relation to the regulatory mechanism.



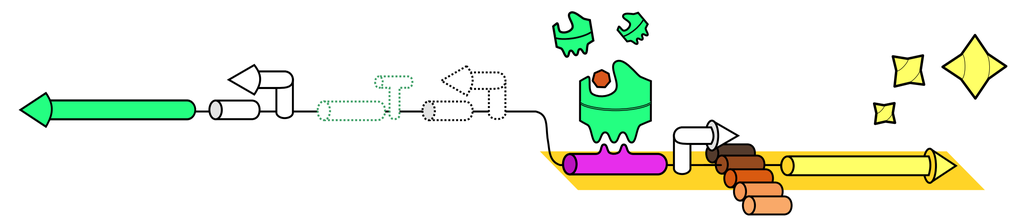
Hill function

$$f(I) = \frac{FP_{cor}}{OD_{cor}} = k(a + \frac{[I]^n}{[I]^n + K_M^n})$$

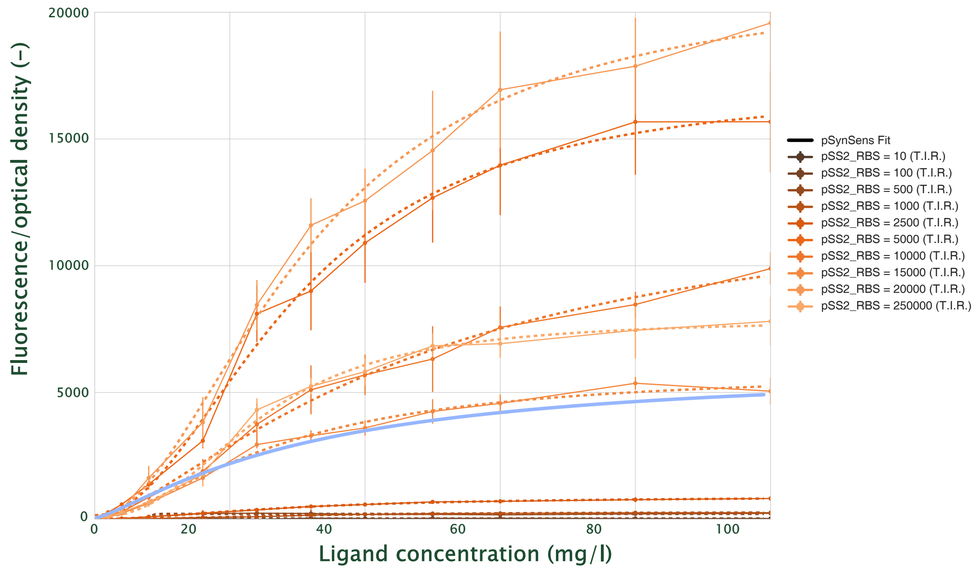
$[I]$ = Concentration of the ligand molecule
k = The maximum normalized fluorescent signal
a = The basal normalized fluorescent signal
K_M = Hill constant (half-maximal ligand concentration)
n = Hill coefficient (cooperativity)

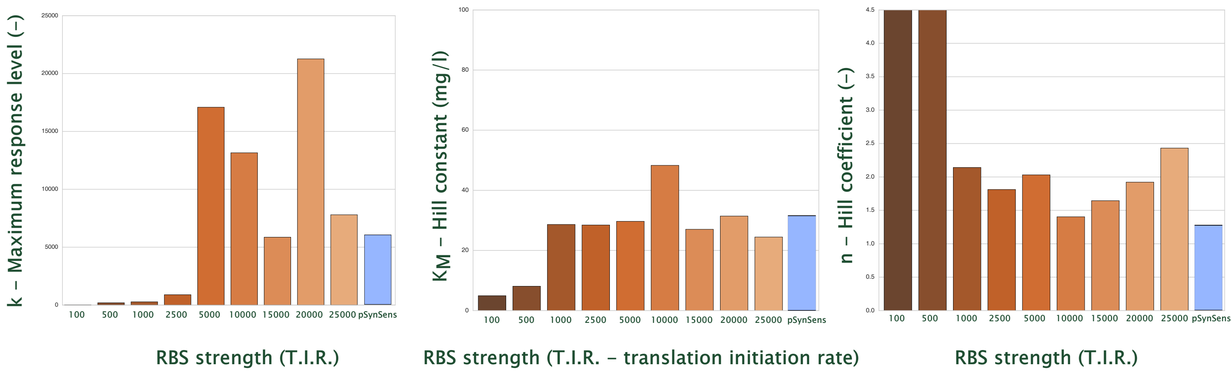


pSynSens2.X – Engineering the effector module



Akin to the detector module, varying the RBS strength of the effector module resulted in a set of custom biosensors with differing characteristics. Noteworthy, when targeting the effector module, a wider range of maximum response levels, with twofold higher maximum response levels, was achieved in comparison to engineering the detector module.





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